Message

From: Jerry Campbell [JCampbell@ramboll.com]
Sent: 11/6/2019 6:34:33 PM
To: Schlosser, Paul [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=121cf759d94e4f08afde0ceb646e711b-Schlosser, Paul]
Subject: RE: Chloroprene PBPK: Peer review charge questions
Attachments: Fmouse_InVivo_SA.R

Paul,

After discussion with Harvey and a few others just to get a consensus, CV would be the best option for the in vivo study simulation. It's most likely somewhere in between depending on how the heart is punctured but we have always used mixed venous for plotting as you noted. I've only used arterial in one model and it was to simulate difference in arterial and venous plasma concentrations collected simultaneously in humans. I've created the separate parameter file for female mouse metabolism which calls mouse.R and then replaces metabolic constants for the female. I've made the change in my version of the model. I also added the parameter value to the output file for the SA analysis (see example). That'll make it easier to calculate the coefficients in excel without having to copy over the parameters separately.

Jerry Campbell

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From: Schlosser, Paul <Schlosser.Paul@epa.gov>
Sent: Monday, November 4, 2019 2:20 PM
To: Jerry Campbell <JCampbell@ramboll.com>
Subject: RE: Chloroprene PBPK: Peer review charge questions

See if attached (copied below) isn't easier to QA. Using CV as the output, the absolute values output to mouseinvivo_sa.csv are different, ~ 15 lower than CVLUM, but the SCs are pretty close to what was in the spreadsheet.

Note I've created two supplemental scripts to avoid re-checking initial model loading and param value setting every time they are used. … If you had to revise one of the female mouse metabolic parameters, how many places to you need to remember to make the corresponding change?

-Paul

```
# Simulates the 15 day mouse exposure study
# Data collected during and after exposure on 1st day
# and at end of exposure on day 5 and 15 (1 day nose-only)
#Uses Table 3 metabolism rates

source("initialize.R")  # script that loads dll, etc.

#Scenario specific values
```

```
tstart <- 0.0
tstop <- 5.95
times <- seq(tstart, tstop , by=0.05)
nend=length(times)

#load the parameters
source('./params/Mouse.R')  # Physiolgical parameters
source('./states.R')

# timing variables for forcing functions
dstart <- tstart
dlength <- 6.0     #hours per day to expose
ddaysperwk <- 5  #days of week to expose
dexpend <- 19    #days of exposure
parms["TSTOP"] <- tstop

# Source forcing functions
# this loads the function forcing() in the namespace
source("forfunc.R")

source('./params/Fmouse_parms.R') # script with default female mouse metabolic parameters

#Scenario Specific Parameters
parms["BW"]<-  0.022   #measured in the study
parms["QPC"]<-  37.6   #measured in the study
parms["QCC"]<-  25.9   #V/Q Ratio Marino et al. 2006

#Scenario Specific Exposure

cs=c(12.3, 32.0, 90.0)  # exposure concentrations (ppm)

pname <- c("BW", "QPC", "QCC", "QLC", "QFC", "QSC", "QKC",
          "VLC", "VLUC", "VFC", "VRC", "VSC", "VKC",
          "PL", "PLU", "PF", "PS", "PR", "PB", "PK",
          "VMAXC", "KM", "VMAXCLU", "KMLU", "KFKIC" )
pval <- parms[pname]  # vector with baseline values of params in pname
pout <- array(0,c(length(pname)+1,3)) # empty results array, +1 row for base values
colnames(pout)<- c("12.3 ppm", "32.0 ppm","90.0 ppm")
rownames(pout)<- c("base",pname)  # first row is base values, then names in pname

for(i in 1:3){
   # Run base model
   parms["CONC"]<-  cs[i]
 out <- ode(Y, times, func = "derivs", parms = parms, method="vode",  atol=1.0e-10, rtol=1.0e-8,
           dllname = mName, initforc="initforc", forcings=forcings,
           initfunc = "initmod", nout = length(Outputs),
           outnames = Outputs)

   pout["base",i] <- out[nend, "CV"]  # first row is base values, *CV* is variable analyzyed

   # run model with each of pval varied, then re-set
 for(pn in pname){
   parms[pn] <- pval[pn]*1.01

   out <- ode(Y, times, func = "derivs", parms = parms, method="vode",  atol=1.0e-10, rtol=1.0e-8,
             dllname = mName, initforc="initforc", forcings=forcings,
             initfunc = "initmod", nout = length(Outputs),
             outnames = Outputs)

   pout[pn,i] <- out[nend, "CV"]
   parms[pn] <- pval[pn]  # reset param to base value
  }
```

```
}
pout <- pout*1000/parms["MW"] # convert units to uM

#unload the model dll
dyn.unload(paste0(mName,.Platform$dynlib.ext))

write.csv(pout,file='mouseinvivo_sa.csv')
```

From: Jerry Campbell <JCampbell@ramboll.com>
Sent: Monday, November 04, 2019 11:12 AM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Subject: RE: Chloroprene PBPK: Peer review charge questions

Where is QCC wrong? Are you sure you are reviewing the correct version of the model? Where did you get a script that plots CVLUM vs gas uptake blood concentration? The final version of the model transmitted with the report had no provision for running gas uptake. There are only 4 simulation scripts in the final model (listed below). If you have additional scripts or model code, they do not belong with this model or report as I have no idea what the parameters are set to or whether or not they have been QC'd at all with regards to the final model. If you are attempting to use a previous version of the model with current parameters, that outside what was transmitted and would need to be created and QC'd before you attempt QA on it.

Fmouse_InVivo.R
Fmouse_metric.R
Human_Continuous.R
Human_Metric.R

I'm fine with one file that is Fmouse_parms.R but you specifically did not like that format in the initial review where you wanted a single physiological parameter script for the species and sex specific parameters set separately. You are more attuned to how reviewer will run the model.

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From: Schlosser, Paul <Schlosser.Paul@epa.gov>
Sent: Friday, November 1, 2019 9:36 PM
To: Jerry Campbell <JCampbell@ramboll.com>
Subject: RE: Chloroprene PBPK: Peer review charge questions

You still didn't put QCC right in one place. This is horrendously more complicated than it needs to be, and each time you write out the default params in another place in another script, you create more opportunity for a QA error. Just make one script with FMouse_params (the metabolic ones specific to the Fmouse) and call it each time needed. Then only that file needs to be

checked. And why keep resetting study-specific params, when all you have to do is vary the ones being evaluated for sensitivity one by one?

Anyway, I see that the script which is supposed to create the plot of CV vs. the gas uptake blood data is actually plotting CVLUM, and CVLU is *not* equal to CV, they differ by ~ 20%. And the SA script is using CV without converting to uM units, while the spreadsheet you sent is in uM units.

This looks like it's going to be a *long* QA.

-Paul

From: Jerry Campbell <JCampbell@ramboll.com>
Sent: Friday, November 01, 2019 12:46 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Subject: RE: Chloroprene PBPK: Peer review charge questions

Try this one. It's because QCC is still 20.1 in the pval list for the loop. I was having issues with resetting parameters after they were run in the for loop. When I added the source for parameter file it changed QCC back to the base model value of 20.1 so I commented it out to test and never corrected it like QPC. This one was more of a pain to set up than I expected just due to housekeeping. The first iteration generated a cumulative 1% change in parameters.

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From: Schlosser, Paul <Schlosser.Paul@epa.gov>
Sent: Friday, November 1, 2019 11:48 AM
To: Jerry Campbell <JCampbell@ramboll.com>
Subject: RE: Chloroprene PBPK: Peer review charge questions

I'm going to avoid copying everyone on this one;

In Fmouse_InVivo_SA.R, why is this one commented out? Doesn't look right, since the relatively high study-specific QPC is in use.

#parms["QCC"]<- 25.9 #V/Q Ratio Marino et al. 2006

From: Schlosser, Paul
Sent: Friday, November 01, 2019 11:28 AM
To: Jerry Campbell <JCampbell@ramboll.com>; Harvey Clewell <HClewell@ramboll.com>
Cc: Walsh, Patrick <patrick-walsh@denka-pe.com>; Thayer, Kris <thayer.kris@epa.gov>; Cascio, Wayne <Cascio.Wayne@epa.gov>; Jones, Samantha <Jones.Samantha@epa.gov>; Lavoie, Emma <Lavoie.Emma@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Kirby, Kevin <KIRBY.KEVIN@EPA.GOV>; Vandenberg, John <Vandenberg.John@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; White, Paul <White.Paul@epa.gov>; Hawkins, Belinda <Hawkins.Belinda@epa.gov>
Subject: RE: Chloroprene PBPK: Peer review charge questions

Sorry, I did see where earlier in the report it's stated that female mice were used for the in vivo study, had been focused on the results section for my QA.

And per our previous emails, the lowest exposure was 12.3 ppm in the in-vivo PK study, so the listing of that concentration in the report text and figures should be corrected from "13 ppm". I suggest using "12.3" in the report, not "12", since it's 12.3 in the model scripts.

-Paul

From: Schlosser, Paul
Sent: Friday, November 01, 2019 11:08 AM
To: Jerry Campbell <JCampbell@ramboll.com>; Harvey Clewell <HClewell@ramboll.com>
Cc: Walsh, Patrick <patrick-walsh@denka-pe.com>; Thayer, Kris <thayer.kris@epa.gov>; Cascio, Wayne <Cascio.Wayne@epa.gov>; Jones, Samantha <Jones.Samantha@epa.gov>; Lavoie, Emma <Lavoie.Emma@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Kirby, Kevin <KIRBY.KEVIN@EPA.GOV>; Vandenberg, John <Vandenberg.John@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; White, Paul <White.Paul@epa.gov>; Hawkins, Belinda <Hawkins.Belinda@epa.gov>
Subject: RE: Chloroprene PBPK: Peer review charge questions

Jerry, Harvey, all,

First QA questions. ☺

In the model report section on plethysmography, the average ventilation measured is 56.2 mL/min in 22 g mice. The QPC is then calculated using an assumed ratio of 2/3 for alveolar/total ventilation, right? I can match 39.4 L/h/bw3/4 given that value. And from that I confirm QCC = 27.2 L/h/bw^0.75, using the ratio of 1.45.

First question: But in Fmouse_InVivo.R, we have:
parms["QPC"]<- 37.6 #measured in the study
parms["QCC"]<- 25.9 #V/Q Ratio Marino et al. 2006
Where does QPC = 37.6 come from? (The QCC corresponds to V/Q = 1.45, given that.)

The report notes that QCC = 27.2 is close to the value obtained from the Marino DCM study, 24.2. But 27.2 is a lot larger than the value used for internal metric calculations, 20.1. Is it reasonable to expect that animals in a nose-only system will have cardiac output (and respiration) 35% higher than those in tox study exposures?

Second, Table S-1 lists BWs for mice and rats as 0.03 and 0.25 kg, citing Brown et al., same weight for males and females. But the IVIVE calculations (Supp D) use 0.035 and 0.04 kg for female and male mice, and 0.33 and 0.45 kg for female and male rats, respectively. The discrepancy vs. default value range from 17% to 50%. Are these study-specific BWs, and if so, which specific study? I think they should be the BWs for the animals from which the tissues were collected for the in vitro studies, but this should be stated in the spreadsheet comments. However, I looked but didn't see BWs in Himmelstein et al.

The _metric scripts use the default BW values in Table S-1… So BW^0.75 scaling is then being used to scale metabolism to the standard BW used to calculate the internal metrics, or 22 g of the animals used in the in vivo dosimetry study. (Those were female mice? This isn't said in the report.)

Note: if we have study-specific BW values for the tox studies being analyzed, those should be used rather than default BWs. I don't think this needs to be done in the report, since what's being shown are example calculations, we aren't at the point of full risk extrapolation. But if EPA does use the model, study-specific animal BWs would be more appropriate.

-Paul

From: Jerry Campbell <JCampbell@ramboll.com>
Sent: Wednesday, October 30, 2019 11:24 AM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>; Harvey Clewell <HClewell@ramboll.com>
Cc: Walsh, Patrick <patrick-walsh@denka-pe.com>; Thayer, Kris <thayer.kris@epa.gov>; Cascio, Wayne <Cascio.Wayne@epa.gov>; Jones, Samantha <Jones.Samantha@epa.gov>; Lavoie, Emma <Lavoie.Emma@epa.gov>;

Bahadori, Tina <Bahadori.Tina@epa.gov>; Kirby, Kevin <KIRBY.KEVIN@EPA.GOV>; Vandenberg, John <Vandenberg.John@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; White, Paul <White.Paul@epa.gov>; Hawkins, Belinda <Hawkins.Belinda@epa.gov>
Subject: RE: Chloroprene PBPK: Peer review charge questions

Paul,

You were correct. The sensitivity runs had been completed in the acslX version and the files had not been included. I've created the scripts that run each of the three sensitivity simulations and a spreadsheet that includes the SA coefficients calculations and bar charts for the report. The zip file name is chloroprene_model_SA.zip and you can download it from the dropbox folder where we shared the model files. The scripts should run in the version of the model you have. Let me know if you have any questions or if something was off when you tried to run the files.

Regards,

Jerry Campbell

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From: Schlosser, Paul <Schlosser.Paul@epa.gov>
Sent: Friday, October 11, 2019 4:58 PM
To: Jerry Campbell <JCampbell@ramboll.com>; Harvey Clewell <HClewell@ramboll.com>
Cc: Walsh, Patrick <patrick-walsh@denka-pe.com>; Thayer, Kris <thayer.kris@epa.gov>; Cascio, Wayne <Cascio.Wayne@epa.gov>; Jones, Samantha <Jones.Samantha@epa.gov>; Lavoie, Emma <Lavoie.Emma@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Kirby, Kevin <KIRBY.KEVIN@EPA.GOV>; Vandenberg, John <Vandenberg.John@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; White, Paul <White.Paul@epa.gov>; Hawkins, Belinda <Hawkins.Belinda@epa.gov>
Subject: RE: Chloroprene PBPK: Peer review charge questions

Jerry, Harvey,

I'm not seeing scripts to run the sensitivity analyses, results in Figures 6 and 7, and plot in Figure 8. None of these is very hard, but things add up. Do you have those?

Also, in Table 1 and the small table on p. 21, the lowest mouse exposure should be 12.8 ppm, not 12.3, right?

-Paul

From: Jerry Campbell <JCampbell@ramboll.com>
Sent: Wednesday, October 09, 2019 11:54 AM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>; Harvey Clewell <HClewell@ramboll.com>
Cc: Walsh, Patrick <patrick-walsh@denka-pe.com>; Thayer, Kris <thayer.kris@epa.gov>; Cascio, Wayne <Cascio.Wayne@epa.gov>; Jones, Samantha <Jones.Samantha@epa.gov>; Lavoie, Emma <Lavoie.Emma@epa.gov>;

Bahadori, Tina <Bahadori.Tina@epa.gov>; Kirby, Kevin <KIRBY.KEVIN@EPA.GOV>; Vandenberg, John <Vandenberg.John@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; White, Paul <White.Paul@epa.gov>; Hawkins, Belinda <Hawkins.Belinda@epa.gov>
Subject: RE: Chloroprene PBPK: Peer review charge questions

Paul,

You are absolutely correct. The figure you derived from the model is the correct figure for the female mouse in vivo study. I had hoped to determine the source of the incorrect figure but I do not have a version of the model going back to Yuching's original model that generates the figure included in the report so I am at a loss to explain the origin. It is my fault it wasn't updated in the final report. I have corrected the report figure but it sounds like we should hold off on sending you the revision in case any other questions need to be addressed as you complete your QA.

Regards,

Jerry Campbell

Managing Consultant

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From: Schlosser, Paul <Schlosser.Paul@epa.gov>
Sent: Monday, October 7, 2019 1:34 PM
To: Harvey Clewell <HClewell@ramboll.com>; Jerry Campbell <JCampbell@ramboll.com>
Cc: Walsh, Patrick <patrick-walsh@denka-pe.com>; Thayer, Kris <thayer.kris@epa.gov>; Cascio, Wayne <Cascio.Wayne@epa.gov>; Jones, Samantha <Jones.Samantha@epa.gov>; Lavoie, Emma <Lavoie.Emma@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Kirby, Kevin <KIRBY.KEVIN@EPA.GOV>; Vandenberg, John <Vandenberg.John@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; White, Paul <White.Paul@epa.gov>; Hawkins, Belinda <Hawkins.Belinda@epa.gov>
Subject: RE: Chloroprene PBPK: Peer review charge questions

Harvey, Jerry, all,

I assume the draft charge questions have been sent to you by now. I realized there's a mistake in the 2nd question in the section titled, "Estimation of Metabolic Parameters from In Vitro Metabolism Experiments." I had thought that the data (and model parameters) used were from male mice (data in original Himmelstein papers, 2004), but I now realize these are female mouse data! I've also run into an issue with reproducing those model results.

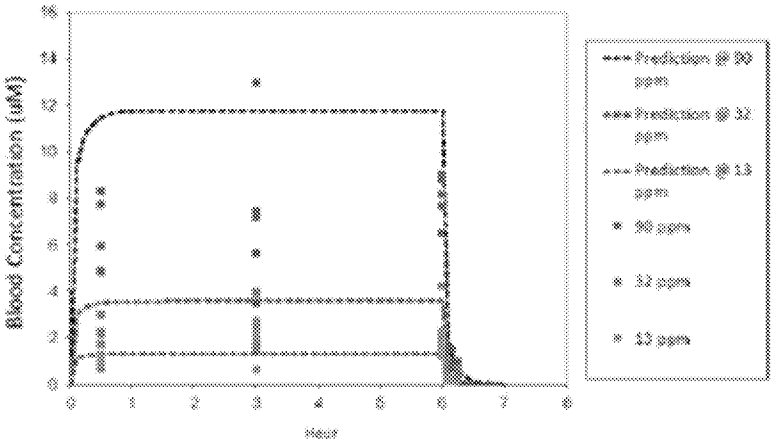
A bit more of an introduction to the question may be helpful, pointing out that these are female data and predictions, but I thought also that the review could be helped by showing predictions for both male and female mouse parameters, to demonstrate the impact of the sex difference.

Setting up to create such a plot, I have hit upon a QA issue: the results I get using the script Fmouse_InVivo.R do not match those shown in Figure 4 of the report. First below is Figure 4 (left panel), 2nd is the plot I first got from just running Fmouse_InVivo.R (files dated 7/16/2019), and third includes dashed lines with predictions using male mouse metabolic parameters. The simulations I'm getting with the package are a lot higher than those shown in the report, which look more like Excel plots in format.

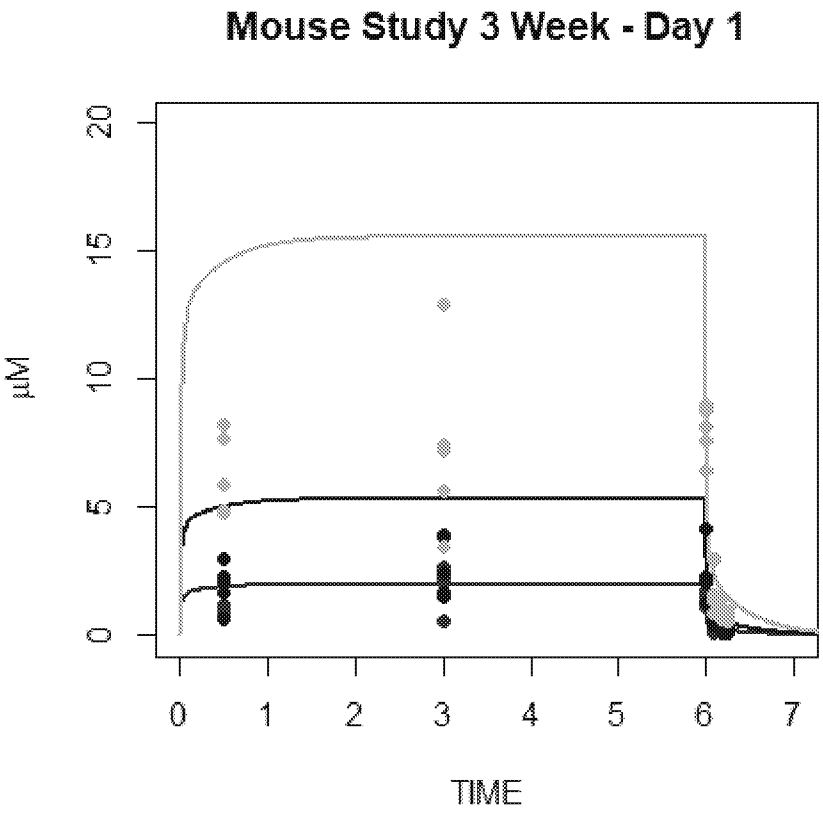
Our intention was to do the QA as the peer review contract was being set up. I'll have to complete my review as soon as possible, so we can resolve any such discrepancies before the material is sent to the reviewers.

-Paul

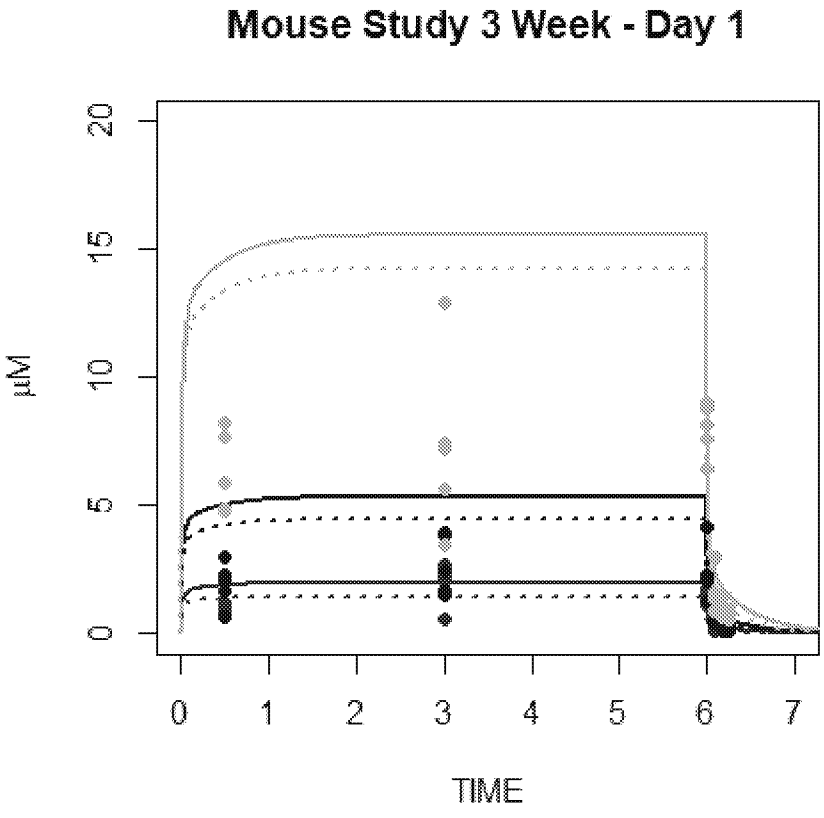
Figure 4 (left panel) from report:



Fmouse_InVivo.R:



Fmouse_InVivo.R with simulations using male mouse parameters (dashed lines) added:



From: Vandenberg, John <Vandenberg.John@epa.gov>
Sent: Wednesday, October 02, 2019 4:46 PM
To: Walsh, Patrick <patrick-walsh@denka-pe.com>
Cc: Thayer, Kris <thayer.kris@epa.gov>; Cascio, Wayne <Cascio.Wayne@epa.gov>; Jones, Samantha <Jones.Samantha@epa.gov>; Lavoie, Emma <Lavoie.Emma@epa.gov>; Schlosser, Paul <Schlosser.Paul@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Kirby, Kevin <KIRBY.KEVIN@EPA.GOV>
Subject: Chloroprene PBPK: Peer review charge questions
Importance: High

Patrick,

We've been diligently evaluating the Ramboll report and conducting analyses related to physiologically-based pharmacokinetic parameters and modeling of chloroprene (references below).

We are moving forward to arrange through a contractor an independent letter peer review by appropriate experts.

In addition to the Ramboll report, we are providing an EPA analysis that we wish to have peer reviewed.

Per our discussion early this summer, we are providing for your information the attached draft Charge questions that will be addressed by the peer reviewers, plus an EPA analysis that we have developed.

Please let us know within a week (by next Wednesday) if you have any major comments on the Charge questions. We are not seeking any comments on the EPA analysis.

Thank you,

John

John Vandenberg, PhD
Director, Health and Environmental Effects Assessment Division
Center for Public Health and Environmental Assessment/ORD
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Research Triangle Park, NC 27711
(919) 541-4527

References:

Ramboll. (2019). Incorporation of in vitro metabolism data in a physiologically based pharmacokinetic (PBPK) model for chloroprene.

U.S. EPA. (2019). In Vitro to In Vivo Extrapolation (IVIVE) of Metabolism and Non-Enzymatic Conjugation of (1-chloroethenyl)oxirane (1-CEO) and Estimation of Total 1-CEO Clearance in the Liver and Lung of Mice, Rats, and Humans.